[Ca\(^{2+}\)]_i oscillations regulate type II cell exocytosis in the pulmonary alveolus

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Ashino, Yugo, Xiaoyou Ying, Leland G. Dobbs, and Jahar Bhattacharya. [Ca\(^{2+}\)]_i oscillations regulate type II cell exocytosis in the pulmonary alveolus. Am J Physiol Lung Cell Mol Physiol 279: L5–L13, 2000.—Pulmonary surfactant, a critical determinant of alveolar stability, is secreted by alveolar type II cells by exocytosis of lamellar bodies (LBs). To determine exocytosis mechanisms in situ, we imaged single alveolar cells from the isolated blood-perfused rat lung. We quantified cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]_i) by the fura 2 method and LB exocytosis as the loss of cell fluorescence of LysoTracker Green. We identified alveolar cell type by immunofluorescence in situ. A 15-s lung expansion induced synchronous [Ca\(^{2+}\)]_i oscillations in all alveolar cells and LB exocytosis in type II cells. The exocytosis rate correlated with the frequency of [Ca\(^{2+}\)]_i oscillations. Fluorescence of the lipidophilic dye FM1-43 indicated multiple exocytosis sites per cell. Intracellular Ca\(^{2+}\) chelation and gap junctional inhibition each blocked [Ca\(^{2+}\)]_i oscillations and exocytosis in type II cells. We demonstrated the feasibility of real-time quantifications in alveolar cells in situ. We conclude that in lung expansion, type II cell exocytosis is modulated by the frequency of intercellularly communicated [Ca\(^{2+}\)]_i oscillations that are likely to be initiated in type I cells. Thus during lung inflation, type I cells may act as alveolar mechanotransducers that regulate type II cell secretion.

cytosolic calcium concentration; surfactant; intercellular communication; fura 2; LysoTracker Green; FM1-43

ALVEOLAR SURFACANT, which reduces the alveolar air-liquid interfacial tension, is critical for normal lung function (reviewed in Refs. 8, 30). Surfactant phospholipids and proteins are stored in the lamellar bodies (LBs) of alveolar type II cells. After stimulation, surfactant phospholipids released by LB exocytosis insert into the alveolar air-liquid interface where their major function is to reduce air-liquid surface tension and thereby promote alveolar stability. Surfactant components may also be protective because surfactant proteins enhance the degradation of lipopolysaccharide by alveolar macrophages (39). Failure to secrete surfactant results in respiratory distress syndrome of the newborn (31). Inactivation of surfactant components in lung injury exacerbates pulmonary edema and alveolar collapse (14).

Lung expansion, a potent stimulus for surfactant secretion (8, 30), may be an important physiological factor in maintaining surfactant production. Hildebran et al. (16) reported that lung expansion for 15 s causes prolonged surfactant secretion lasting >30 min. The basis of this prolonged secretory response to a relatively brief stimulus remains unexplained. It is proposed that alveolar distension attributable to lung expansion causes stretch-induced secretion in type II cells (8, 30, 40). However, real-time determinations of relevant cellular events have not been obtained in the intact alveolus.

The cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]_i) is an important second messenger in secretory processes. During exocytosis, several proteins and small GTPases regulate membrane fusion events leading up to vesicle docking on the cell membrane. This is followed by a final Ca\(^{2+}\)-dependent step that activates release of vesicular contents (33, 38). Ca\(^{2+}\)-dependent mechanisms are implicated in surfactant exocytosis because Ca\(^{2+}\)-ionophores induce secretion (34, 36), Ca\(^{2+}\) is required for LB fusion and aggregation (37), and Ca\(^{2+}\) increases are associated with stretch- or secretagogue-induced secretion in cultured type II cells (17, 40). In a recent report, Haller et al. (16) used flash photolysis of caged Ca\(^{2+}\) to demonstrate induction of [Ca\(^{2+}\)]_i-dependent LB exocytosis. However, not all forms of exocytosis require Ca\(^{2+}\) (5), and Ca\(^{2+}\)-independent processes of surfactant secretion have also been reported (30).

In addition to a direct Ca\(^{2+}\) effect on exocytosis, cell-cell communication of Ca\(^{2+}\) signals across gap junctions provides a mechanism for multicellular regulation of secretion (19, 32). Gap junctions exist between alveolar type I and type II cells (3). Gap junctional inhibitors block cell-cell flow of intracellularly injected dye among cultured type II cells (26). However, the functional significance of intercellular com-
munication in the alveolus remains unknown. Here we consider this in the context of surfactant secretion.

Reported studies of surfactant secretion in the lung are based on bulk methods such as lung lavage that are not amenable to cellular determinations (e.g., Ref. 18). However, new optical methods provide an opportunity for real-time cellular quantifications in situ. In cultured type II cells, surfactant secretion has been determined through quantification of intracellular fluorescence of the acidotropic dye LysoTracker Green (LTG) that accumulates in LBs (17). Another potentially useful agent is the lipophilic exocytosis marker FM1-43 (4), which is weakly fluorescent in aqueous medium but becomes intensely fluorescent during exocytosis as the dye gains access to phospholipids in LBs (16, 29).

Recently, Kuebler et al. (24) identified pressure-induced fusion pore formation in lung capillaries using LysoTracker Green (LTG) and FM1-43 dyes in conjunction with fluorometric [Ca\(^{2+}\)] determinations. Here, we report the first application of the lipophilic exocytosis marker FM1-43, Hoechst 33342 (all from Molecular Probes, Eugene, OR), and trypan blue from Sigma (St. Louis, MO). The antibodies used were cell surface-recognizing mouse anti-rat monoclonal antibodies against alveolar type I (11) or type II (10) cells, anti-integrin antibody LM609 (Calbiochem, La Jolla, CA), and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (ICN Biomedicals, Costa Mesa, CA). The surfactant was 3% reconstituted bovine surfactant (Surfactant, Ross) in Ringer lactate. The blockers were 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetra(acetoxyethyl) ester (BAPTA-AM; Calbiochem), heptanol, histamine (both from Sigma), and halothane (Halocarbon Laboratories, River Edge, NJ).

**Lung Fluorescence Microscopy**

The methods have been previously described (43). Briefly, lungs excised from anesthetized Sprague-Dawley rats were perfused with 14 ml/min of autologous rat blood at 37°C. Pulmonary arterial and left atrial pressures were held at 10 and 5 cmH\(_2\)O, respectively. At baseline, the lungs were constantly inflated at an alveolar pressure (PA) of 5 cmH\(_2\)O. The lung was positioned on a vibration-free table and superfused with normal saline at 37°C to prevent drying. Fluorophores were excited by mercury lamp epi-illumination. Fluorescence was detected with the image-analysis system of Ying et al. (43) consisting of a fluorescence microscope (BH2-RFCA, Olympus America, Melville, NY), an image intensifier (KS1381, Video Scope International, Sterling, VA), a video camera (CCD-72, Dage-MTI, Michigan City, IN), and software for digital image analysis (MCID-M4, Imaging Research, St. Catherine’s, ON).

**Alveolar Microinjection**

For each imaged alveolus, microscope focus was set at the maximum alveolar diameter. By micropuncture, solutions were microinjected into an alveolus to fill three to four neighboring alveoli. Although we did not directly determine injection volume, we expect that each microinjection delivered ~1 nl. We microinjected the solutions gently, and to the extent possible, we determined that no alveolar distension occurred during microinjection. A nonmicropunctured alveolus was selected for imaging measurements. Alveolar microinjections were used for the following protocols.

**Identification of type I and type II cells by indirect immunofluorescence.** Cell surface-recognizing monoclonal antibodies (50 μg/ml) against alveolar type I (11) or type II (10) cells were microinjected separately for 15 min each, and then FITC-conjugated IgG was microinjected as the secondary antibody. Unbound fluorescence in the alveolus was washed by microinjection of 3% reconstituted bovine surfactant in Ringer lactate. Control data were obtained with the secondary antibody alone or with a nonspecific anti-rat primary antibody (LM609) followed by the secondary antibody.

**Alveolar [Ca\(^{2+}\)] determinations.** Our methods for [Ca\(^{2+}\)] quantification were previously described (43). Briefly, membrane-permeant fura 2-AM, which deesterifies intracellularly to the cell-impermeant fura 2, in DMSO-HEPES buffer was microinjected for 30 min. The alveolus was washed as in Identification of type I and type II cells by indirect immunofluorescence and then excited at 340 and 380 nm at 10-s intervals. Epithelial [Ca\(^{2+}\)] was determined from the computer-generated 340- to 380-nm ratio based on a dissociation constant of 224 nmol/l and appropriate calibration parameters (24, 43). All determinations were obtained at a PA of 5 cmH\(_2\)O.

**Type II cell exocytosis.** The exocytosis rate was determined with LTG (50 nmol/l). Sites of exocytosis were determined with FM1-43 (4 μM). The dyes were dissolved in DMSO-HEPES buffer and were microinjected for 15 min and 10 s, respectively. Images were obtained after alveolar washout as in Identification of type I and type II cells by indirect immunofluorescence.

**Ca\(^{2+}\) chelation.** The membrane-permeant Ca\(^{2+}\) chelator BAPTA-AM (30 μM) in Ca\(^{2+}\)-free buffer (composition in mM: 150 NaCl, 5 KCl, 10 glucose, and 20 HEPES, pH 7.4) was microinjected for 15 min, beginning 1 min after lung expansion.

**Gap junctional blockade.** Heptanol (3 mM) or halothane (4 mM) in HEPES solution was injected.

**Miscellaneous agents.** Trypan blue (5% solution in HEPES) and histamine dihydrochloride (10 μM) in HEPES were injected for 1 min each.

**Experimental Protocols**

Sequential imaging for cell-specific quantification. All images were obtained at a PA of 5 cmH\(_2\)O, with the focus set at the plane of maximum alveolar diameter. In each alveolus, we first obtained the fura 2 or LTG images, then we photobleached the fluorescence and identified type I and type II cells by indirect immunofluorescence.

**Lung expansion protocol.** For lung expansion, we increased PA from a baseline of 5 to 15 cmH\(_2\)O for 5–15 s, then we returned PA to baseline. In the isolated rat lung, such an increase in PA simulates an increase of lung volume from end expiratory to 90% of inspiratory reserve volume (13). Consistent with previous reports (23, 42), our lung expansion procedure increased alveolar circumference by 18.5 ± 2.7% (P < 0.05).

**Statistics**

All data are means ± SE. Differences between groups were tested by paired t-test for 2 groups and the Newman-Keuls test for >2 groups. Significance was accepted at P < 0.05.
RESULTS

Fluorescence Images of Fura 2-Loaded Alveoli

The fura 2-loaded alveolus imaged as a circular fluorescent band that circumscribed a central nonfluorescent lumen. In the 340- and 380-nm images of fura 2-loaded alveoli, well-defined spots of high fluorescence were evident as depicted by the green and purple spots in the 340- and 380-nm images, respectively, in Fig. 1A. These spots colocalized with the fluorescence of the nuclear staining dye Hoechst 33324 (Fig. 1B). Similar to the capillary experiments by Ying et al. (43), this colocalization identified the high fluorescence sites as locations of single cells for which the corresponding location in the 340- to 380-nm ratio image gave the [Ca^{2+}]_i value.

Not shown are results from experiments in which we determined that the fluorescence of fura 2 was intracellular because it was not diminished by the fluorescence-quenching agent trypan blue. No detectable leakage of fura 2 occurred from cells because the Ca^{2+}-insensitive fluorescence of fura 2 determined at an excitation of 360 nm remained unchanged for 1 h. Similar to the capillary findings of Ying et al. (43), membrane permeabilization with 5-min intra-alveolar injections of digitonin resulted in complete loss of alveolar fluorescence. This ruled out the presence of residual fluorescence that could have resulted from lysosomal uptake of fura 2. Manganese chloride completely quenched fura 2 fluorescence, indicating that the fluorescence of unbound fura 2 did not contribute to the imaged data.

Alveolar [Ca^{2+}]_i Responses

Baseline. As described in METHODS, we first imaged alveoli for fura 2 fluorescence, then we identified type I and type II cells by immunofluorescence as shown in Fig. 2A. Under baseline conditions with lungs held at a constant Pa of 5 cmH_2O, [Ca^{2+}]_i was 30% higher in type II than in type I cells (Fig. 2B). At a constant Pa, baseline [Ca^{2+}]_i signals were steady in all alveolar cells as indicated by the unchanging pseudocolor in timed images (Fig. 3A).

Lung expansion. A 15-s lung expansion caused pronounced [Ca^{2+}]_i oscillations as exemplified by phasic pseudocolor changes that occurred simultaneously in all cells of the alveolus as shown, for example, in Fig. 3B. Although no consistent increases occurred in mean [Ca^{2+}]_i, the induced [Ca^{2+}]_i oscillations persisted for >30 min in both type I and type II cells (Fig. 4A). Figure 4A, top box, shows that the oscillations were synchronous between the cell types. Similar results were obtained when lung expansions were held for 5 or 10 s. As shown in Fig. 4A, bottom box, mean amplitudes and frequencies of [Ca^{2+}]_i oscillations determined in the first 5 min after the inflation challenge were not different for lung expansions held for 15, 10, or 5 s.

The oscillations were dominated by waves of amplitude > 30 nmol/l that progressively decreased in frequency (Fig. 4B) but not in amplitude (data not shown). A 15-min alveolar microinfusion of the intracellular Ca^{2+} chelator BAPTA-AM given 1 min after lung expansion markedly reduced the frequency of [Ca^{2+}]_i oscillations of amplitude > 30 nmol/l (Fig. 4B). The mean amplitudes of these waves before (35 ± 5 nmol/l) and after (30 ± 4 nmol/l) BAPTA-AM were not significantly different. The effect of giving a similar infusion...
of BAPTA-AM before lung expansion was to inhibit the oscillations completely. This result is not shown because the postexpansion oscillation frequency was not different from baseline (n = 4 alveoli).

Heptanol, which uncouples gap junctions (7), had no effect on baseline \([\text{Ca}^{2+}]_i\). A 15-min intra-alveolar infusion of heptanol given before a 15-s lung expansion desynchronized (Fig. 4C, top tracing) but did not block postexpansion \([\text{Ca}^{2+}]_i\) oscillations in the type I cell (Fig. 4D, compare solid bars). In contrast, heptanol completely blocked \([\text{Ca}^{2+}]_i\) oscillations in the type II cell (Fig. 4, C, bottom tracing, and D, compare open bars). These results indicate that in the type I cell, \([\text{Ca}^{2+}]_i\) oscillations were independently evoked, whereas in the type II cell, the oscillations were communicated from adjoining cells.

Not shown are results from experiments in which histamine increased type II cell \([\text{Ca}^{2+}]_i\) in heptanol-treated alveoli \((P < 0.05; n = 3)\), indicating the absence of nonspecific effects of heptanol on \(\text{Ca}^{2+}\) mobilization. Type II cell \([\text{Ca}^{2+}]_i\) oscillations induced by a 15-s lung expansion \((n = 3\) alveoli) were also blocked by the gap junctional uncoupler halothane (15). Furthermore, two successive lung expansions separated by 40 min elicited identical \([\text{Ca}^{2+}]_i\) oscillations \((n = 3\) alveoli), indicating that the response could be regenerated by repeated mechanical stimulation. These findings support the interpretation that heptanol inhibited type II cell \([\text{Ca}^{2+}]_i\) oscillations by blocking gap junctions.

Exocytosis

Type II cell exocytosis was quantified by the LB marking dye LTG (17). Sequential imaging in which we first imaged LTG-loaded alveoli and then confirmed cell phenotype by immunoimaging (see METHODS) indicated the presence of brightly fluorescent LTG-loaded type II cells (Fig. 5A). As exemplified in Fig. 5B (0 min), the fluorescence was diffuse but nonhomogeneous throughout the cell, consisting characteristically of highly fluorescent, apically distributed spots that presumably represented LB clusters (Fig. 5B, arrowheads).

A single 15-s lung expansion resulted in a progressive decrease of overall cell fluorescence, indicating onset of exocytosis in the type II cell (Fig. 5B). However, as shown in both the images taken at 5–30 min in Fig. 5B and the traces in Fig. 5C, the decrease in fluorescence commenced after varying delays for different spots in the same cell, indicating nonuniformity in the onset of exocytosis. For example, in Fig. 5B, note that some of the apical fluorescent spots were detectable for up to 15 min after the lung expansion challenge. Similarly, Fig. 5C shows that the decrease in fluorescence began almost immediately for one spot (○) but was delayed by 12 min for another (▼). To detect the sites of exocytosis in type II cells, we used the lipidophilic dye FM1-43 (17, 29). The fluorescence of FM1-43 was not detectable at baseline (Fig. 5D, left). However, 20 min after a 15-s lung expansion, FM1-43 fluorescence appeared at several locations on the type II cell (Fig. 5D, right), indicating the formation of multiple exocytotic sites in the same cell.

Under baseline conditions, total LTG fluorescence in the cell decreased 5% from the initial level in 30 min (Fig. 6A, black circles). This slow exocytosis rate is consistent with the baseline surfactant secretion rates reported in vivo (30). After a 15-s lung expansion, fluorescence decreased 90% from the initial level in 30 min (Fig. 6A, blue circles), indicating marked augmentation of exocytosis. The fluorescence decrease was exponential, with a time constant of 16 ± 2 min.

Intra-alveolar heptanol injection completely blocked exocytosis (Fig. 6A, green circles). Similar results were obtained with intra-alveolar injections of halothane.
In heptanol-treated alveoli, intra-alveolar histamine induced exocytosis (Fig. 6B), indicating that heptanol did not block the exocytotic mechanisms of the cell. These findings indicate that the expansion-induced type II cell exocytosis resulted from signals that were intercellularly communicated.

To determine the effects of an induced reduction of \([Ca^{2+}]_i\) oscillations, we injected alveoli with BAPTA-AM in the postexpansion period. This treatment markedly reduced the exocytosis rate (Fig. 6A, red circles). Given before lung expansion, BAPTA-AM completely blocked exocytosis (data not shown; \(n = 4\) alveoli). A combined plot of the postexpansion data at 5-min intervals for both the control and \([Ca^{2+}]_i\) chelation experiments revealed an exponential relationship between oscillation frequency and exocytosis rate (Fig. 7), suggesting that type II cell exocytosis was modulated by \([Ca^{2+}]_i\) oscillations.

**DISCUSSION**

Our findings indicate that in the alveolus, a 15-s lung expansion induced type II cell exocytosis that persisted for up to 30 min. Hence to the extent that type II cell exocytosis indicates surfactant secretion, we confirm the original finding of Hildebran et al. (18) that a brief period of lung expansion causes prolonged surfactant secretion. The lung expansion also induced prolonged \([Ca^{2+}]_i\) oscillations in both type I and type II cells over a time course that corresponded with the duration of exocytosis. The gap junctional inhibitors heptanol and halothane specifically blocked both \([Ca^{2+}]_i\) oscillations and exocytosis in type II cells. The
intracellular Ca$^{2+}$ chelator BAPTA-AM markedly attenuated both effects when given 1 min postexpansion but blocked both effects completely when given preexpansion. The important conclusions from these findings are that lung expansion causes type II cell exocytosis that is [Ca$^{2+}$]$_i$ dependent and that results from intercellular communication of [Ca$^{2+}$]$_i$ oscillations to the type II cell. Because surfactant is the principal exocytosed product of type II cells, these conclusions may apply to lung expansion-induced surfactant secretion.

Because of the three-dimensional alveolar structure, cell-specific alveolar imaging by epifluorescence microscopy required defined landmarking. We obtained all images at a fixed PA, which ensured constant alveolar geometry in both baseline and postexpansion periods, and we maintained focus at a fixed level, namely at the plane of the largest alveolar diameter. Thus to the extent possible, cells were imaged under identical conditions of alveolar geometry and at an identical alveolar plane. Because expansion caused lung motion, we relocated the targeted alveolus or single cell using location landmarks such as vessel bifurcations identified before lung expansion. This protocol enabled us to image cells in specifically targeted alveoli throughout the experimental period. To identify cell phenotype in relation to fluorescence responses, we first imaged cells for the fluorescence of fura 2 or LTG. Then we photobleached these fluorophores to eliminate interference from their fluorescence and carried out a further round of alveolar injections to define cell phenotype by immunofluorescence imaging. These procedures indicate the feasibility of obtaining cell-specific functional data from alveoli in situ.
Our quantification of baseline \([Ca^{2+}]_i\) in the type II cell in situ compares well with previously reported data (22, 40) from cultured type II cells in which resting \([Ca^{2+}]_i\) varies from 107 to 143 nM. However, although in cultured type II cells, cell stretch or soluble surfactant secretagogues cause short-lived \([Ca^{2+}]_i\) transients (29, 40), the present \([Ca^{2+}]_i\) oscillations were sustained for up to 30 min. \([Ca^{2+}]_i\) oscillations are attributed to \(Ca^{2+}\)-dependent ligational properties of endosomal inositol 1,4,5-trisphosphate receptors (12) as well as to oscillatory release of inositol 1,4,5-trisphosphate (20). Although present mechanisms remain unclear, the fact that \([Ca^{2+}]_i\) increased even when lung expansion was held for as briefly as 5 s indicates that during the respiratory cycle, short deep breaths such as sighs may be adequate for generating sustained \([Ca^{2+}]_i\) oscillations.

According to Haller et al. (16), a threshold \([Ca^{2+}]_i\) of 320 nmol/l is required to initiate LB exocytosis in type II cells. In our experiments, mean \([Ca^{2+}]_i\) did not increase significantly, and even the oscillation peaks seldom exceeded 160 nmol/l. However, we may have missed an initial transient that was higher because \([Ca^{2+}]_i\) recordings were begun with a delay of 1–2 min after lung expansion. Because BAPTA-AM given before lung expansion blocked exocytosis, we confirm the finding of Haller et al. that the initiation of exocytosis is \([Ca^{2+}]_i\)-dependent. However, our experiments indicate that \([Ca^{2+}]_i\) played a more persistent role because exocytosis was attenuated by BAPTA-AM given even in the postexpansion period, indicating that \([Ca^{2+}]_i\) oscillations were required for the maintenance of exocytosis.

Although the oscillations contained both low- and high-amplitude waves, we restricted the present analysis to waves of amplitude > 30 nmol/l. For these waves, a combined plot of the data from control and BAPTA-AM-treated conditions revealed an exponential relationship between the time-dependent decreases in LTG fluorescence and \([Ca^{2+}]_i\) oscillation frequency (Fig. 7). This relationship suggests that the type II cell secretion rate was frequency modulated by the \([Ca^{2+}]_i\) oscillations. The biochemical basis of this secretion-frequency relationship may lie in calmodulin kinase II, which is implicated in surfactant secretion (28) and is activated in a frequency-dependent manner by \(Ca^{2+}\) oscillations (9). However, the relevance of...
present oscillation frequencies in calmodulin kinase II activation remains undetermined.

Heptanol, a gap junctional inhibitor, is widely used in studies of intercellular communication. In several cell types (7, 27, 35) as well as in lung capillaries (43), heptanol inhibits cell-cell communication of Ca\(^{2+}\) signals. However, heptanol may have nonspecific effects that block both Ca\(^{2+}\) mobilization and exocytosis. We ruled out these possibilities through three sets of findings. 1) Although heptanol blocked [Ca\(^{2+}\)]\(_i\) oscillations in type II cells, it did not block oscillations in type I cells. Therefore, the type II cell effect was phenotype specific and not the result of nonspecific inhibition. 2) Histamine, a surfactant secretagogue (6), both increased [Ca\(^{2+}\)]\(_i\), and also stimulated exocytosis in type II cells. These effects were not blocked by heptanol, indicating that at the present concentrations, heptanol did not nonspecifically inhibit Ca\(^{2+}\) mobilizing mechanisms or vesicular events associated with exocytosis. 3) Inhibitions similar to heptanol were also replicated by halothane, a gap junctional blocker that is chemically dissimilar from heptanol (7). Taken together, these findings support the present conclusions based on the blocking effect of heptanol on intercellular communication.

Pattern of Secretion

In cultured type II cells exposed to surfactant secretagogues, Haller et al. (17) and Mair et al. (29) reported real-time monitoring of LB exocytosis by quantifying the decrease in fluorescence in LTG-loaded cells and the increase in membrane-associated fluorescence of FM1-43. In our experiments, type II cells fluoresced brightly in LTG-loaded alveoli, whereas fluorescence of type I cells was minor and could be eliminated from the image by reducing the photometer gain (Fig. 6A). In confirmation of findings of Haller et al. (16) and Mair et al. (29), the fluorescence of FM1-43 indicated the appearance of several type II cell exocytosis sites in the postexpansion period. Both the apical distribution of LB clusters, as indicated by fluorescent spots in LTG-loaded cells, and the luminally distributed exocytosis sites were consistent with the expected functional polarity of type II cell exocytosis. Although overall cell fluorescence decreased exponentially, with a time constant similar to that of stimulated cultured type II cells (17), the fluorescence of individual LB clusters decreased nonuniformly (Fig. 5C). These findings indicate that in alveoli, secretion occurs as multiple exocytotic events at different apical sites of the type II cell.

Concluding Remarks

In summary, our findings reveal a novel mechanism by which intercellular communication between alveolar epithelial cells regulates lung expansion-induced secretion in the alveolar type II cell. Intercellular communication is also implicated in ciliary body secretion (19) and bile production (32) and may, in general, be a regulator of secretion in multicellular systems. An important conclusion to be drawn from our studies is that surfactant secretion may not be entirely self-regulated by the type II cell and that substantial regulation may be attributable to communication of [Ca\(^{2+}\)]\(_i\) oscillations from adjoining cells, most likely the type I cell. This interpretation is derived from our findings that [Ca\(^{2+}\)]\(_i\) oscillations were synchronous between type I and type II cells and that gap junctional uncouplers blocked [Ca\(^{2+}\)]\(_i\) oscillations in type II but not in type I cells. Taking these results together, we propose that during alveolar expansion, stretch on the type I cell evokes [Ca\(^{2+}\)]\(_i\) oscillations that are communicated to the type II cell. Secretion then results in a frequency-modulated manner. Thus type I cells may act as alveolar mechanotransducers that modulate type II cell secretion. The relevance to disease may be that gap junctional defects attributable to cell phenotype modifications are being recognized in some forms of lung injury (1, 2, 21, 25, 41). Hence our present proposal subsumes the possibility that pathology of cell communication may underlie abnormalities of surfactant secretion in lung disease. This possibility requires further consideration.

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